Clinical Laboratory Perspective: Current State and Challenges for Clinical Labs Implementing and Offering NGS-based Tests

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CLINICAL TESTING • RESEARCH • EDUCATION

Disclosures

Consultant:

- Illumina
- Strand Analytical Labs

Co-founder:

- PierianDx
- P&V Licensing, LLC

Academic affiliation:

- Washington University School of Medicine is an academic, tertiary care, urban medical center

- GPS is a reference lab, within the Department of Pathology, that performs a range of molecular testing, including NGS

NGS Based Testing at GPS@WUSTL

https://gps.wustl.edu/

- Somatic
  - Comprehensive Cancer Panel (177 genes; disease specific subsets)
  - TCR clonality
  - Myeloseq (40 genes; MRD assay with LLD of 0.1% VAF); launch May 1, 2018
  - Gene Fusion (507 genes); launch late 2018
  - cfDNA within cyst fluid (275 genes); launch late 2018
- Inherited
  - Somatic overgrowth and related syndromes (20 genes; 14 different diseases)
  - Cardiovascular (80 genes; 8 different diseases)
  - Congenital neutropenia (24 genes)
  - Medical renal disease (69 genes; 4 different disease classes)
  - HLA typing

Note the range of assay designs (hybrid capture vs amplification based); platforms (Illumina vs ThermoFisher); nucleic acid sequenced (DNA vs RNA); library preparation method (classical vs UMI-based); bioinformatics (somatic vs inherited vs UMI-based); etc.

"NGS" is a Method, Not a Test

Analytically:

- **Different platforms for "NGS"**
- **Different assay designs for "NGS" (amplification based versus hybrid capture; tumor only versus tumor-normal pairs; somatic versus germline)**
- **Different bioinformatic pipelines**
- **Different assay validation schemes**
- **Different intended uses (e.g., for just direct sequence analysis of one analyte (DNA): solid tumor DNA vs cfDNA vs MRD using UMIs)**
- **Inherited disease testing versus somatic testing**

Do regulatory entities (and payers) understand this?

Current State

- There are no standards based criteria for traditional metrics like sensitivity, specificity, PPV, NPV
- There is no standardization of NGS tests with the same intended use as far as
  - Target regions
  - Test design
- There is no standardization of the bioinformatics pipelines of NGS tests with the same intended use as far as
  - Variant classes detected
  - Reference databases
- There is no standardization of interpretation of NGS tests with the same intended use as far as
  - Criteria
  - Databases
  - Qualifications of lab staff
- There is no agreement on what constitutes the "gold standard" for either inherited disease testing or somatic testing

Current Lab Accreditation Paradigms are Largely "Process Based" versus "Standards Based"

It's pretty clear regulatory entities and payers understand this!

Logical Conclusions

- The classical approaches to test validation *and* laboratory accreditation are not appropriate for the unique aspects of NGS methods
- The range of NGS-based test designs, sequencing platforms, and bioinformatics adds to the challenge
- It's time to consider standards based approaches in addition to process based approaches for test validation and lab accreditation
- It's time to admit that patient care is absolutely dependent on LDTs as well as FDA cleared tests, and will be for the foreseeable future

Uncertainty Regarding Regulatory Approach

- What are the fundamental elements for assay clearance that FDA is looking for? Is it "standards based" transparency?
- The data show that FDA clearance and/or CDx designation do not indicate superior "standards based" test performance either technically or operationally. So what is the basis?
- How broadly or narrowly will regulatory agencies group "intended use" of tests when considering assay clearance and/or CDx designation?

Uncertainty Regarding Test Validation

- Several groups are addressing this problem
  - Six musketeers/ad hoc standards group (sponsored by CAP, with participation of AMP)
  - CAP Genomic Medicine Resource Committee (chaired by Karl Voelkerding).
  - CLSI MM09 project*
- How do we get these groups to, at the least, talk with one another and harmonize recommendations so we're not left with non-overlapping documents?

https://clsi.org/volunteer/volunteer-opportunities/mm09-human-genetic-and-genomic-testing/

Lack of Availability of Reference Materials

"Wet Samples"

Actual tumor samples
- are physiologic
- are not inexhaustible

Cell lines are inexhaustible
- blended genomic DNA samples with pre-defined variant profiles
- soup to nuts
- limited in number of genes, variants, VAFs
- expensive to develop
- time consuming to develop

"Dry Samples" (In Silico Files):

Pre-defined introduced by a computerized process into NGS sequence files
- limited to bioinformatics component of the test
- virtually unlimited flexibility
- inexpensive to create
- quick to create

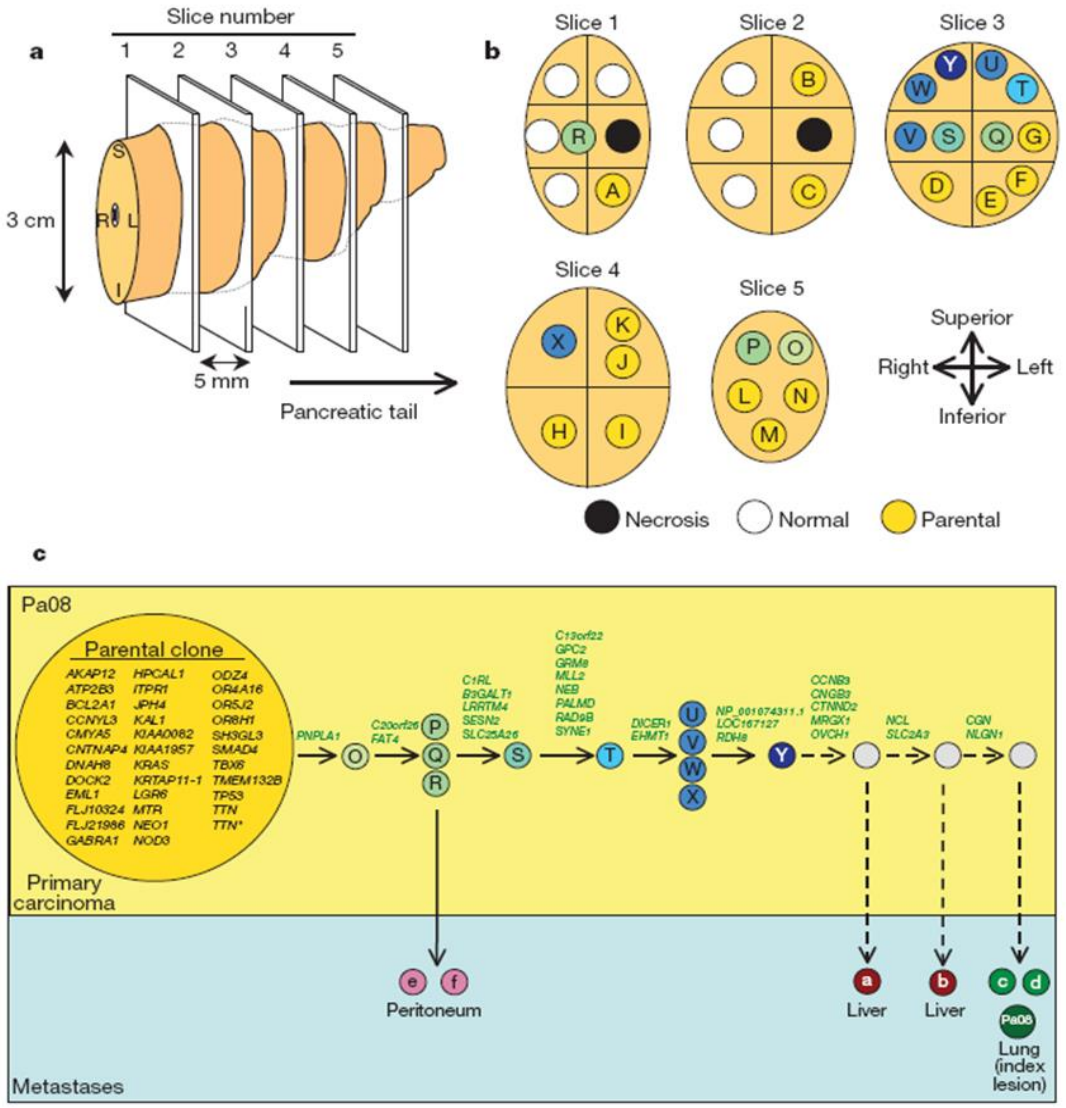
The Quality Assurance Pilot of the Sustainable Predictive Oncology Therapeutics and Diagnostics (SPOT/Dx) Working Group

- **Sustainability:** traceable reference samples (quality control materials) that are commercially maintainable
- **Transparency of results:** visibility of outcomes
- **Accelerated reference material (RM) creation/availability:** appropriate for use in phase 3 of CDx/drug development, prior to market launch
- **Collaborative dialogue:** diversity and balance of perspectives
- **Quick:** test proof of concept as rapidly as possible; evolve process as needed

http://www.tapestrynetworks.com/initiatives/healthcare/oncology-therapeutics-and-diagnostics/index.cfm

Emerging NGS Based Testing Issues

- **Cancer oligoclonality, clonal evolution, and the need to move past single-point NGS testing**
- **Need for sampling multiple areas of the primary tumor, as well as different metastatic foci**
- **ctDNA, RNAseq, TMB, neoantigen epitope prediction**
- **And so on…**



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Implications

The lack of standards (which carries with it the lack of transparency) impacts patient care.

A requirement for regulatory clearance neither ensures the "best" test nor acknowledges the pace of scientific discovery, technical advancement, and clinical applications.

Validation and proficiency testing samples are desperately needed to make possible objective review of lab performance.